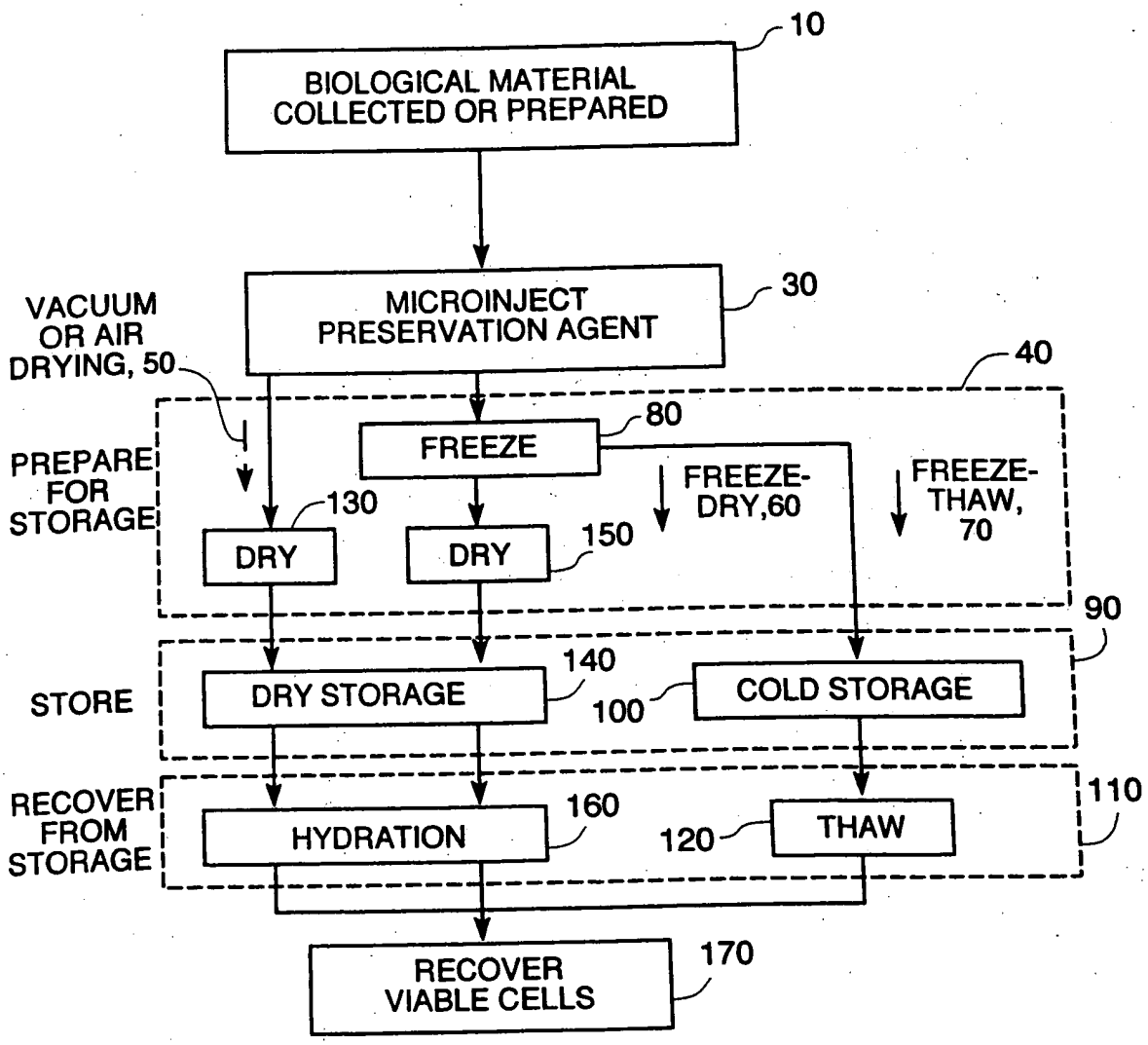
FIG. 1



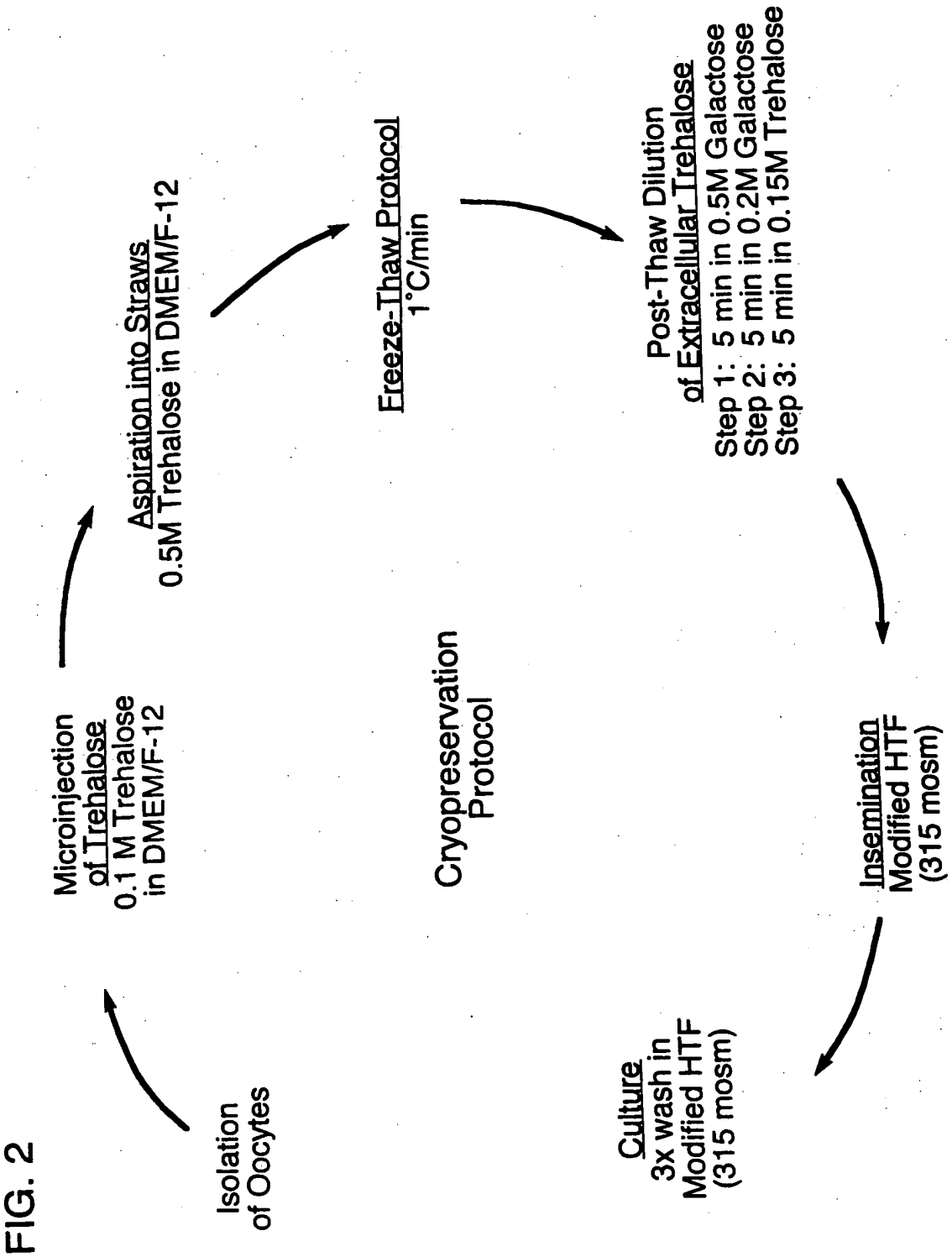

Isolation of Oocytes
Microinjection of Trehalose 0.1 M Trehalose in DMEM/F-12
Aspiration into Straws 0.5M Trehalose in DMEM/F-12
Freeze-Thaw Protocol 1°C/min
Post-Thaw Dilution of Extracellular Trehalose
Step 1: 5 min in 0.5M Galactose
Step 2: 5 min in 0.2M Galactose
Step 3: 5 min in 0.15M Trehalose
Insemination Modified HTF (315 mosm)
Culture 3x wash in Modified HTF (315 mosm)
Cryopreservation Protocol

FIG. 2

FIG. 3A

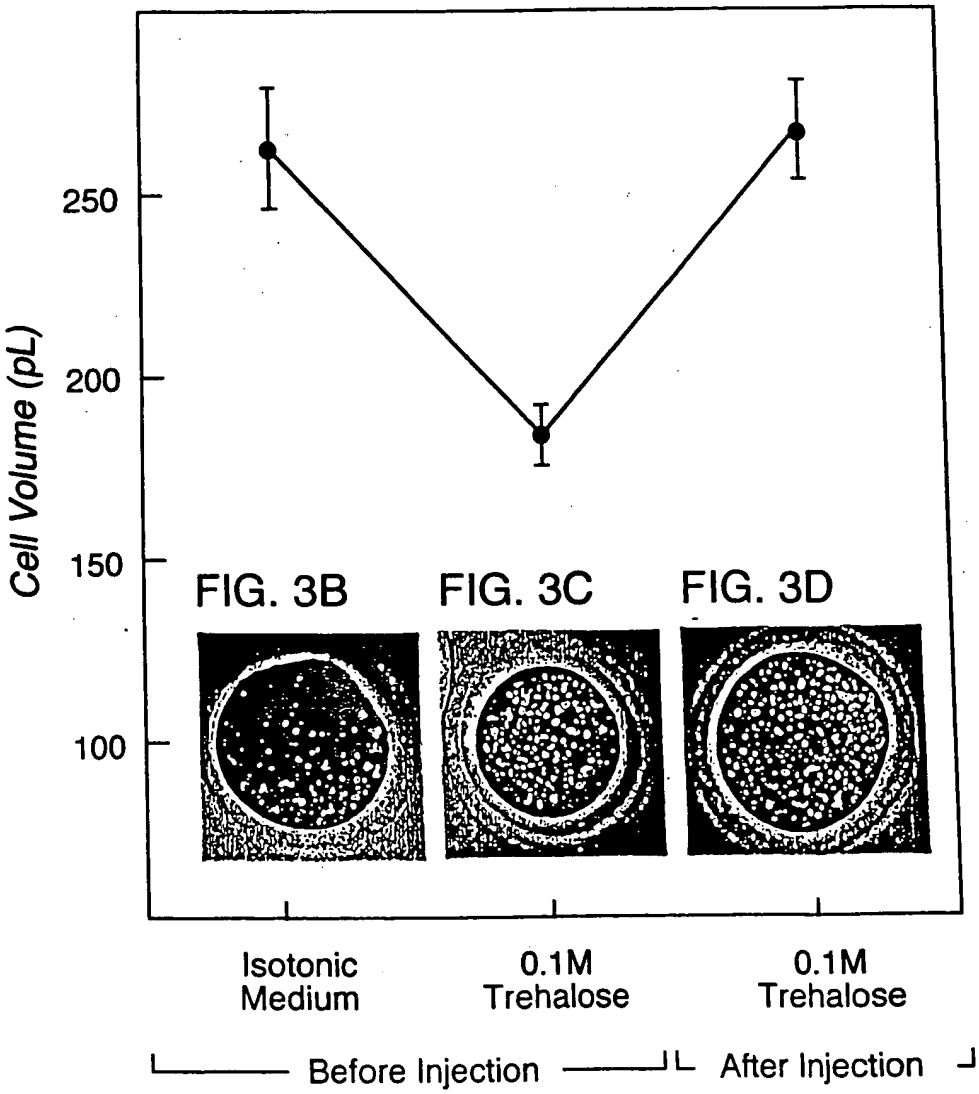

Cell Volume (pL)
250
200
150
100
FIG. 3B
FIG. 3C
FIG. 3D
Isotonic Medium
0.1M Trehalose
0.1M Trehalose
Before Injection
After Injection

FIG. 4A

Isotonic Medium
Before Injection

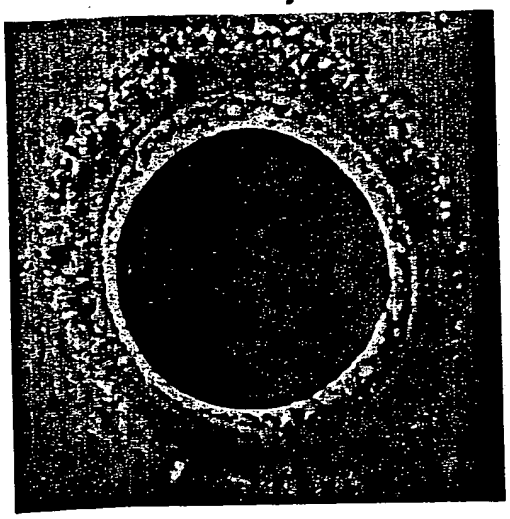


FIG. 4B

0.15M Trehalose
Before Injection

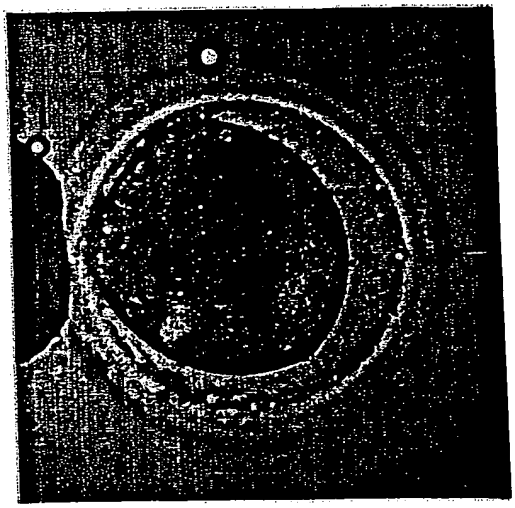


FIG. 4C

0.15M Trehalose
During Injection

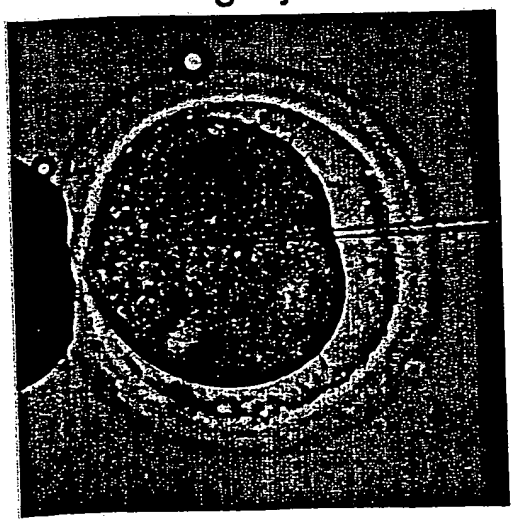
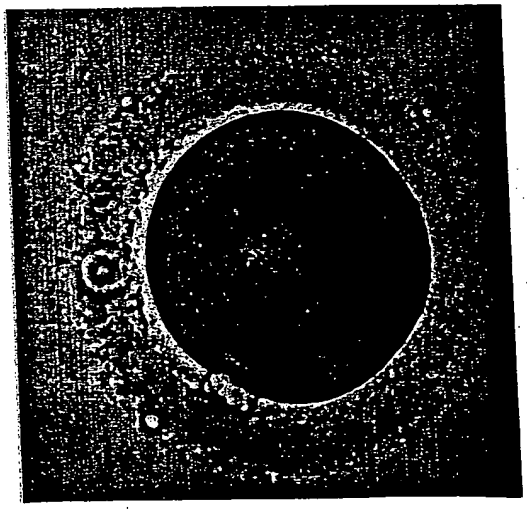


FIG. 4D

0.15M Trehalose
After Injection



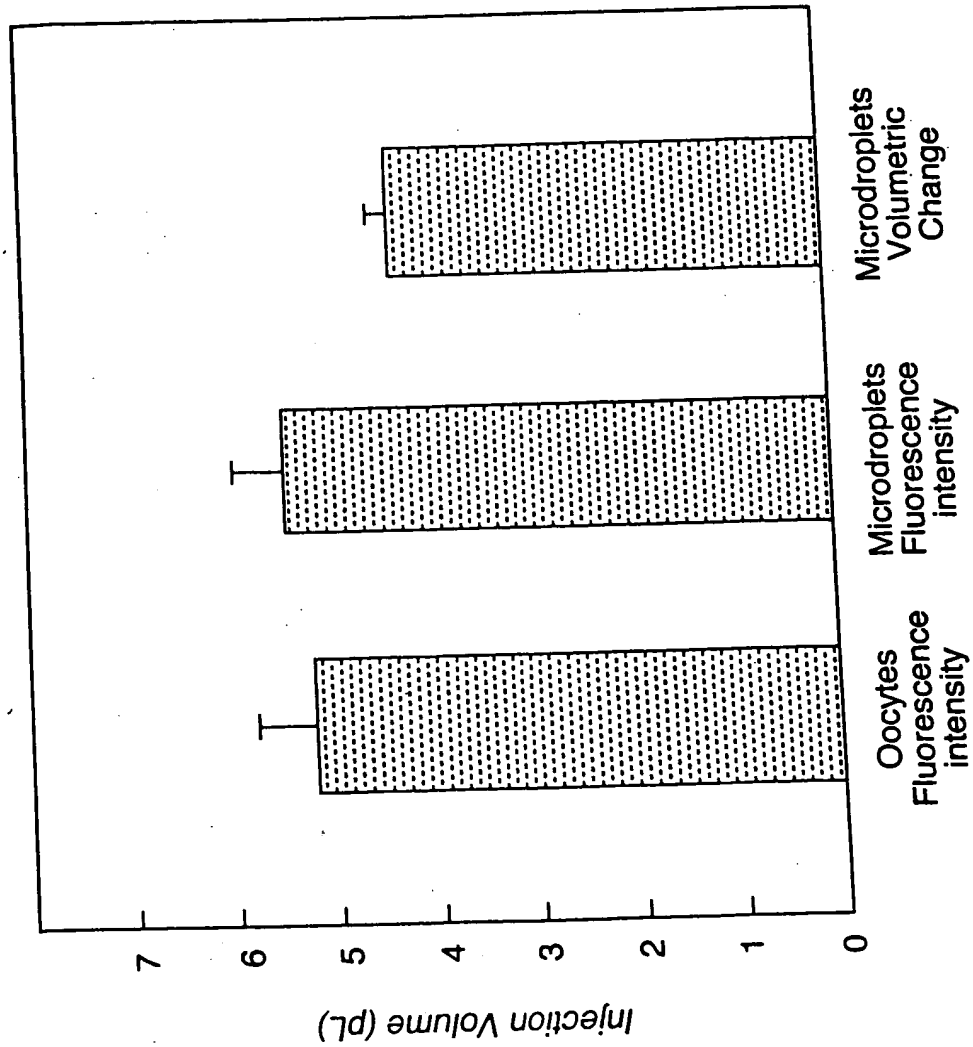

Microdroplets Volumetric Change
Microdroplets Fluorescence intensity
Oocytes Fluorescence intensity
0
1
2
3
4
5
6
7
Injection Volume (pL)

FIG. 5

FIG. 6B

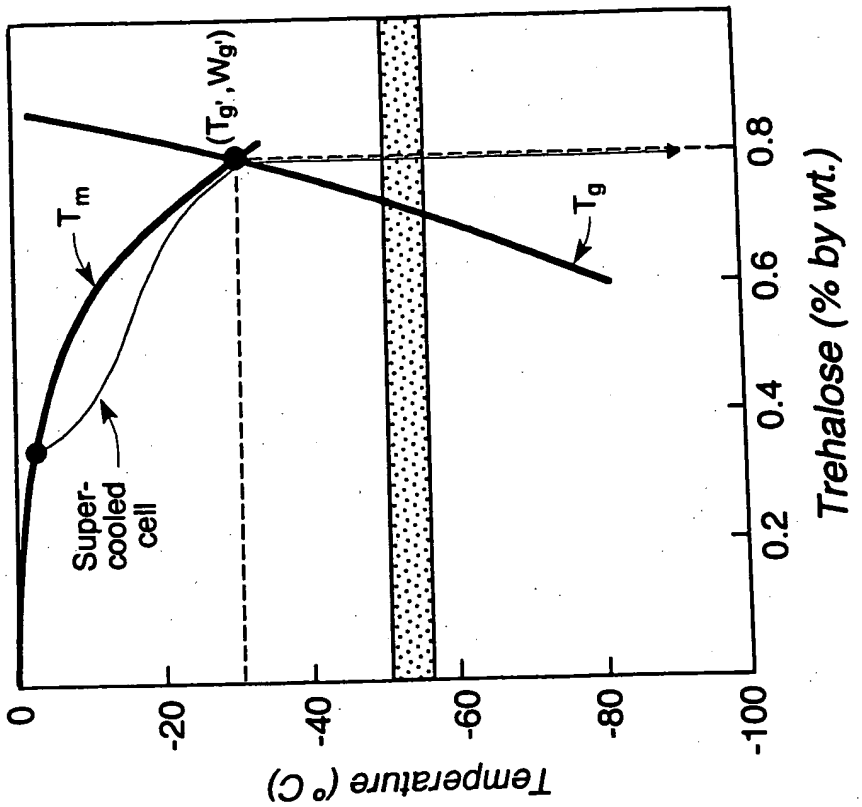

(T_g', W_g')
T_m
T_g
Super-cooled cell
0
-20
-40
-60
-80
-100
Temperature (° C)
0.2
0.4
0.6
0.8
Trehalose (% by wt.)

FIG. 6A

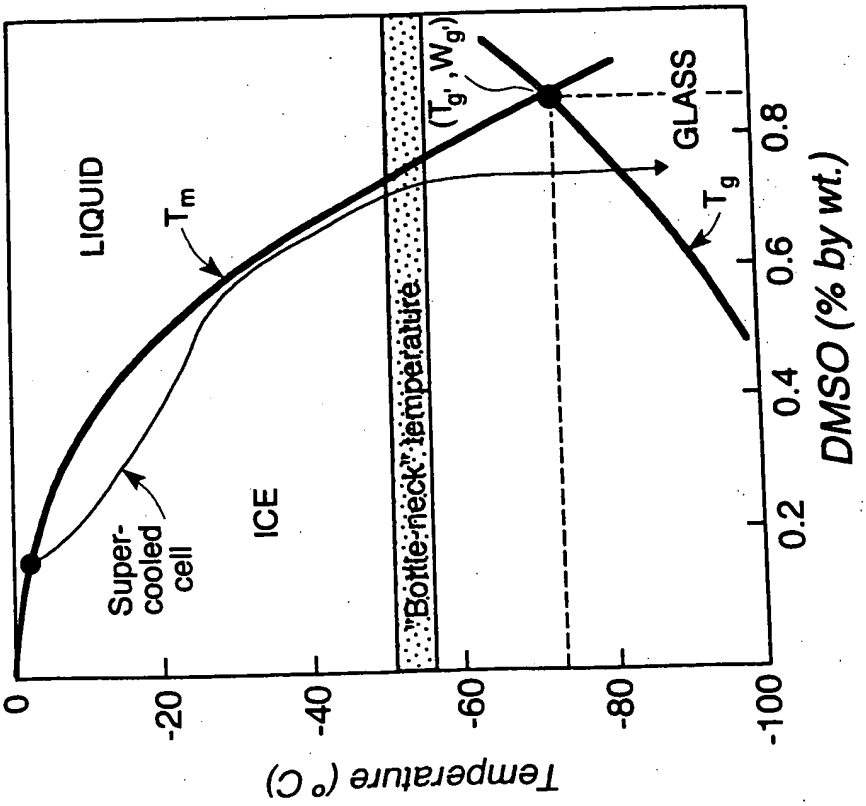

LIQUID
T_m
(T_g', W_g')
GLASS
T_g
Super-cooled cell
ICE
"Bottle-neck" temperature
0
-20
-40
-60
-80
-100
Temperature (° C)
0.2
0.4
0.6
0.8
DMSO (% by wt.)

FIG. 7

Component	HTF	Modified HTF	
		Isotonic	Hypertonic
NaCl (mM)	101.60	▼ 95.00	106.70
KCl (mM)	4.69	▲ 4.78	5.37
KH_2PO_4 (mM)	0.37	▲ 0.38	0.43
$MgSO_4 \cdot 7H_2O$ (mM)	0.20	0.20	0.22
$NaHCO_3$ (mM)	25.00	25.00	28.07
$CaCl_2 \cdot 2H_2O$ (mM)	2.04	▼ 2.00	2.25
Lactate (mM)	21.40	▼ 20.00	22.46
Pyruvate (mM)	0.33	0.33	0.37
D- Glucose (mM)	2.78	2.78	3.12
L-Glutamine (mM)	---	▲ 1.00	1.12
BSA (mg/ml)	4	4.00	4.00
EDTA	---	▲ 0.01	0.01
Essential amino acids	---	▲ 0.5x	0.5x
Non-essential amino acids	---	▲ 0.5x	0.5x
Phenol red (% w/v)	0.01	0.001	0.001
Gentamicin (mg/ml)	50.00	50.00	50.00
Osmolarity (mosm)	**285.00**	**285.00**	**320.00**

FIG. 8

Culture Condition	No. of Experiments	No. of Inseminated Oocytes	Percent Two-cell	Percent Blastocyst
Modified HTF, isotonic	5	58	90% (52/58)	87% (51/58)
Modified HTF, hypertonic	9	234	90% (211/234)	86% (202/234)
$[Tre]^{in}$ = 0.07M	3	32	78% (25/32)	66% (21/32)
$[Tre]^{in}$ = 0.15M	5	69	67% (46/69)	29% (20/69)

FIG. 9A

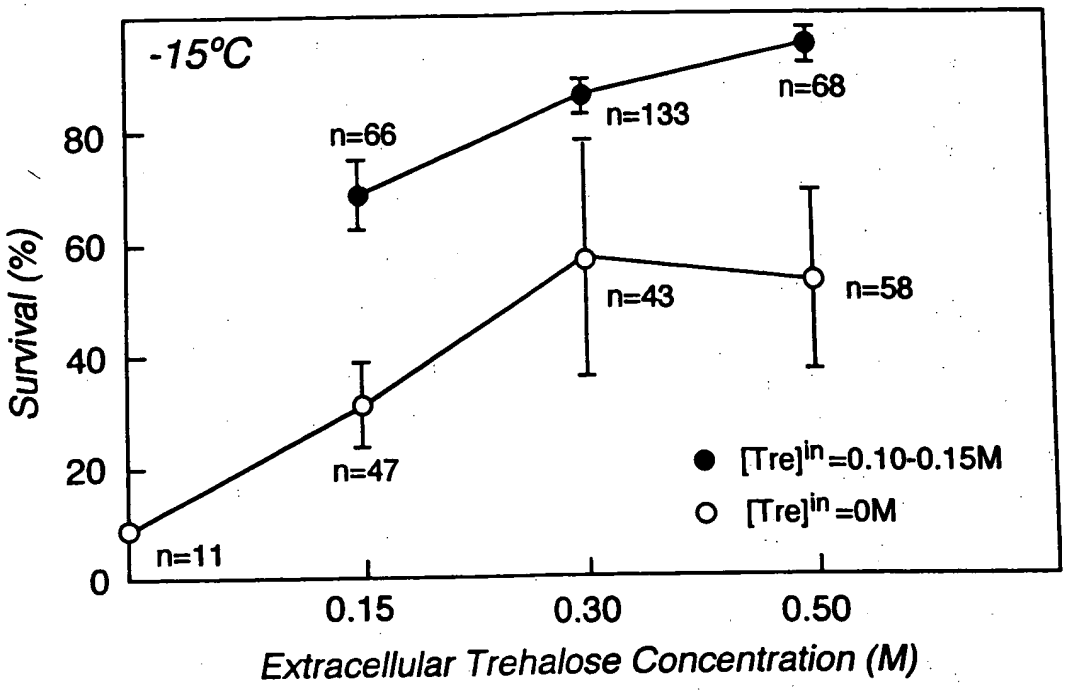

-15°C
Survival (%)
0
20
40
60
80
n=66
n=133
n=68
n=43
n=58
n=47
n=11
[Tre]in=0.10-0.15M
[Tre]in=0M
0.15
0.30
0.50
Extracellular Trehalose Concentration (M)

FIG. 9B

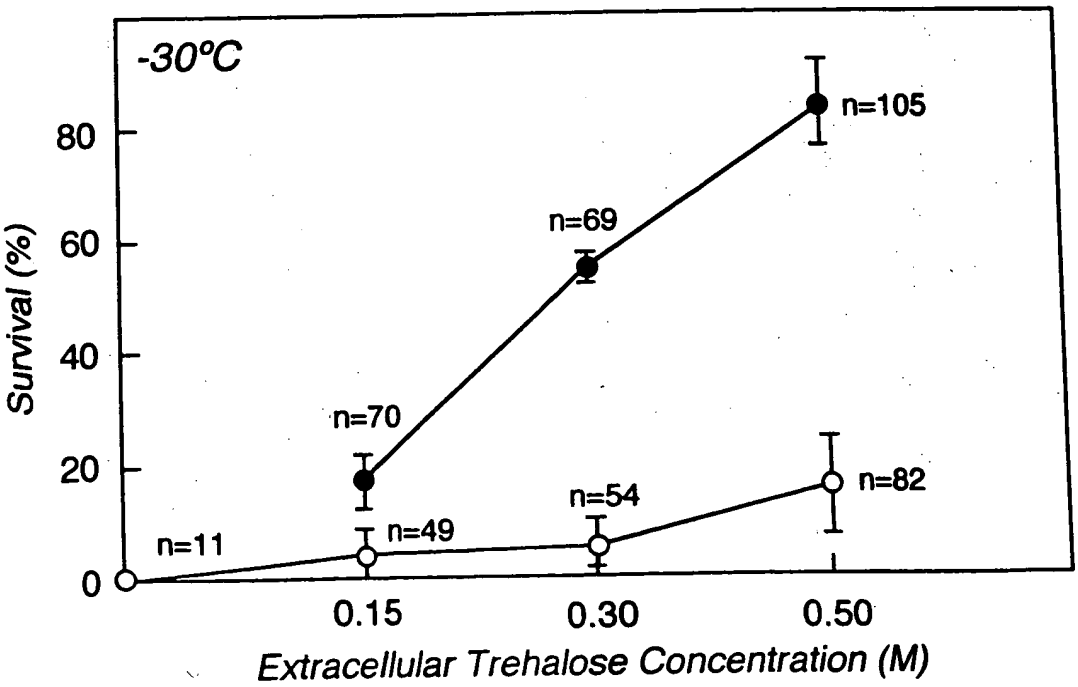

-30°C
Survival (%)
0
20
40
60
80
n=105
n=69
n=70
n=54
n=82
n=49
n=11
0.15
0.30
0.50
Extracellular Trehalose Concentration (M)

FIG. 10

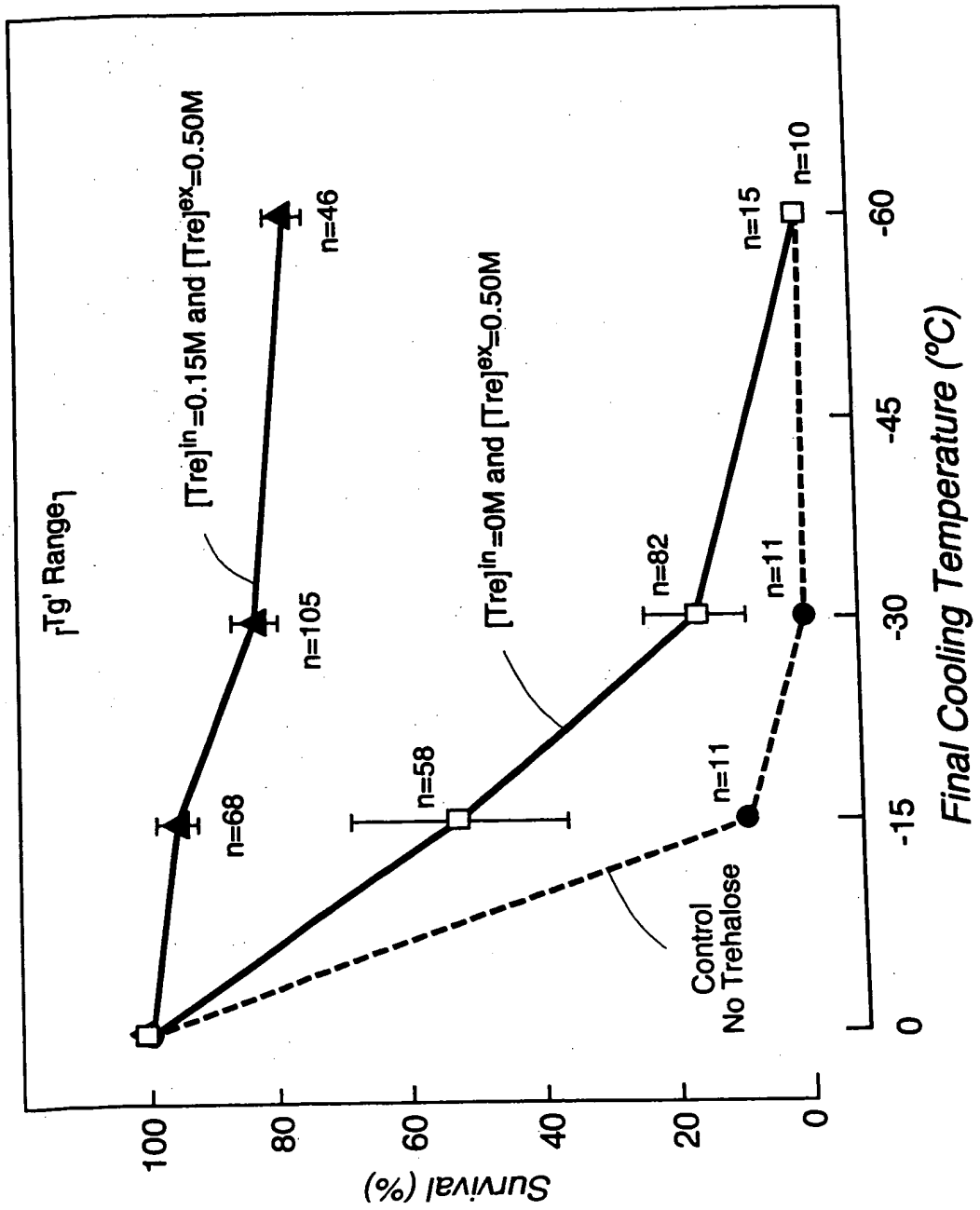

Survival (%)
100
80
60
40
20
0
Final Cooling Temperature (°C)
0
-15
-30
-45
-60
[Tg' Range]
[Tre]in=0.15M and [Tre]ex=0.50M
[Tre]in=0M and [Tre]ex=0.50M
n=68
n=105
n=46
n=58
n=82
n=11
n=15
n=10
n=11
Control
No Trehalose

FIG. 11

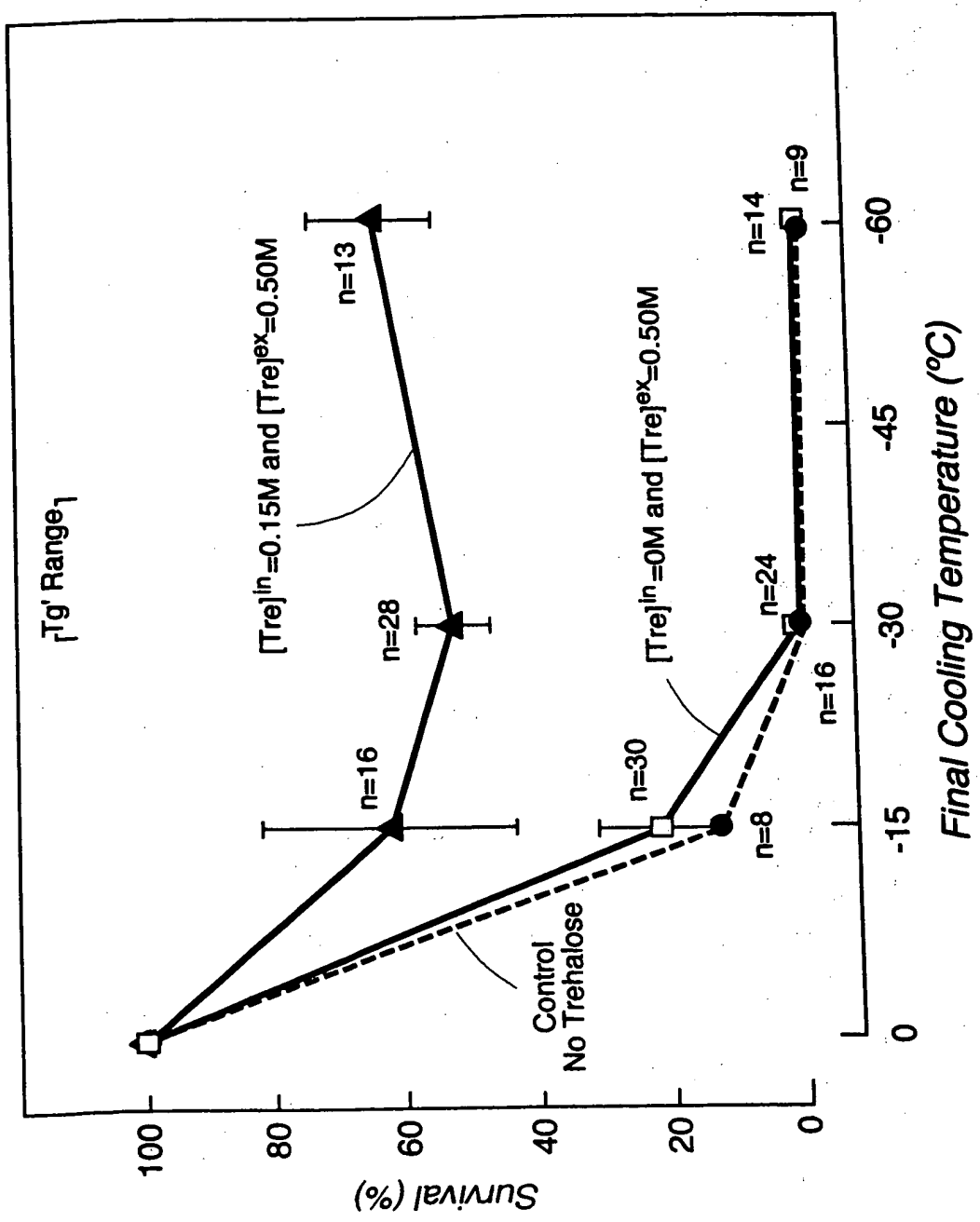

Survival (%)
100
80
60
40
20
0
Final Cooling Temperature (°C)
0
-15
-30
-45
-60
[Tg' Range]
[Tre]^{in}=0.15M and [Tre]^{ex}=0.50M
n=13
n=28
n=16
[Tre]^{in}=0M and [Tre]^{ex}=0.50M
n=14
n=9
n=24
n=16
n=30
n=8
Control
No Trehalose

FIG. 12A

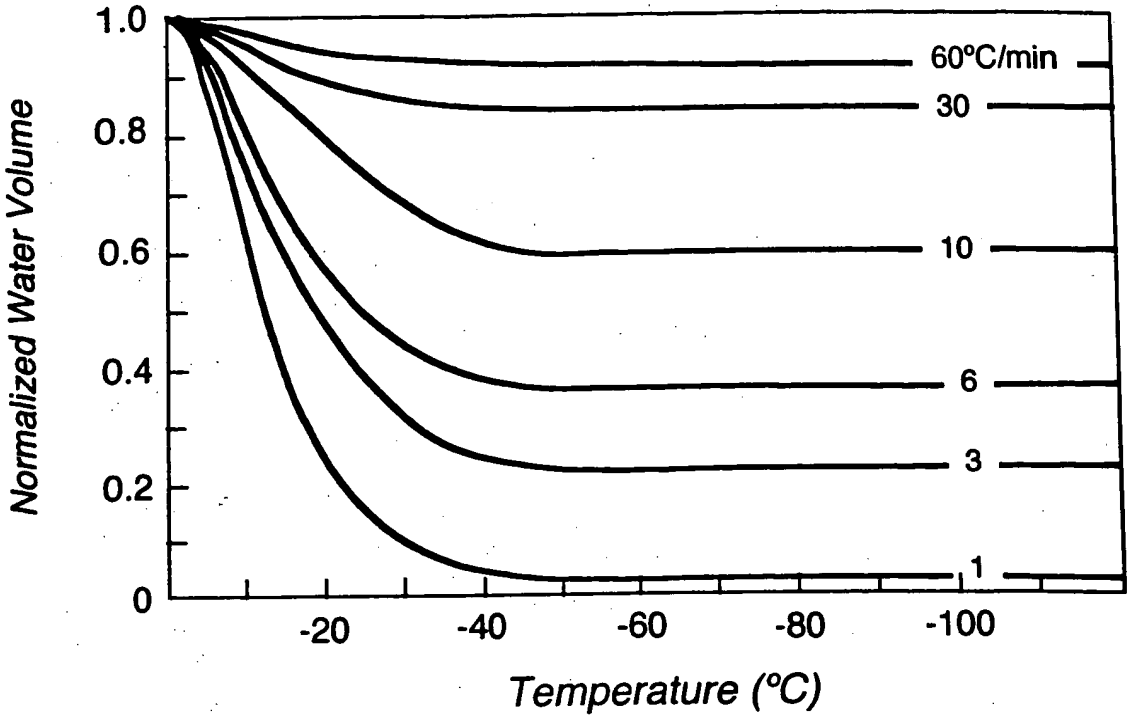
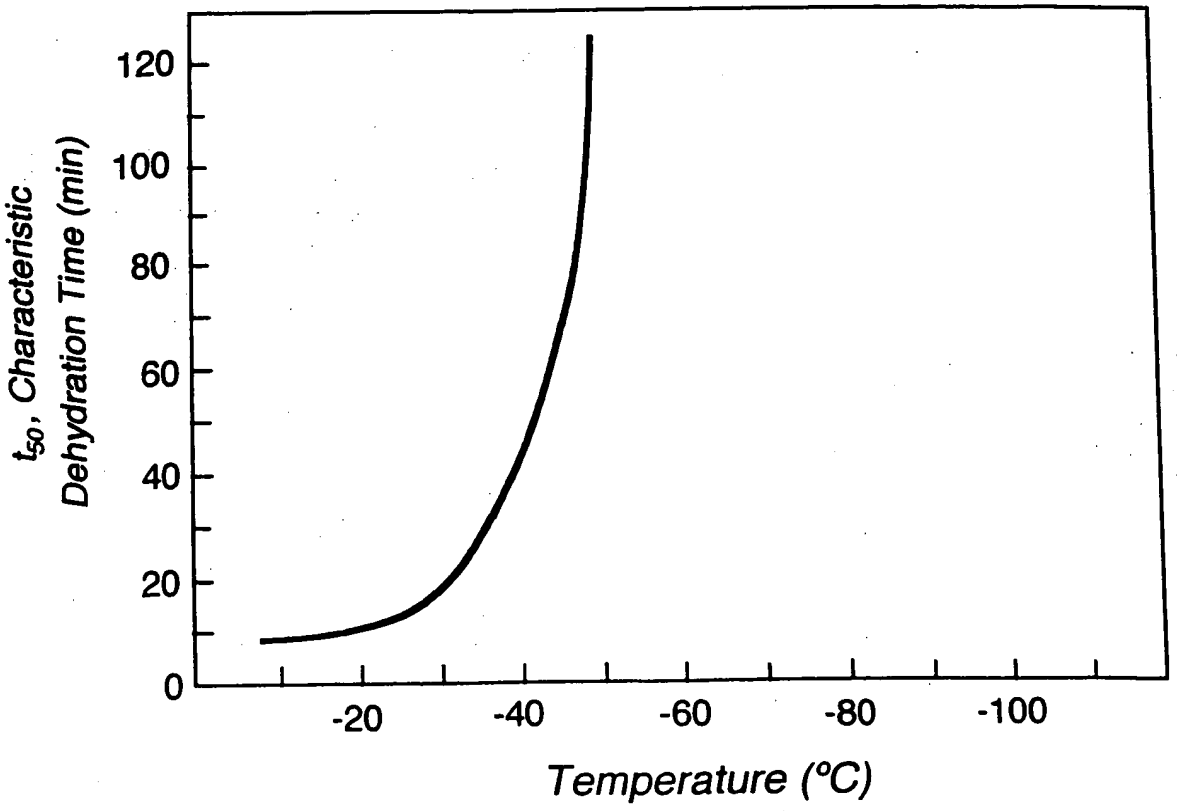


FIG. 12B



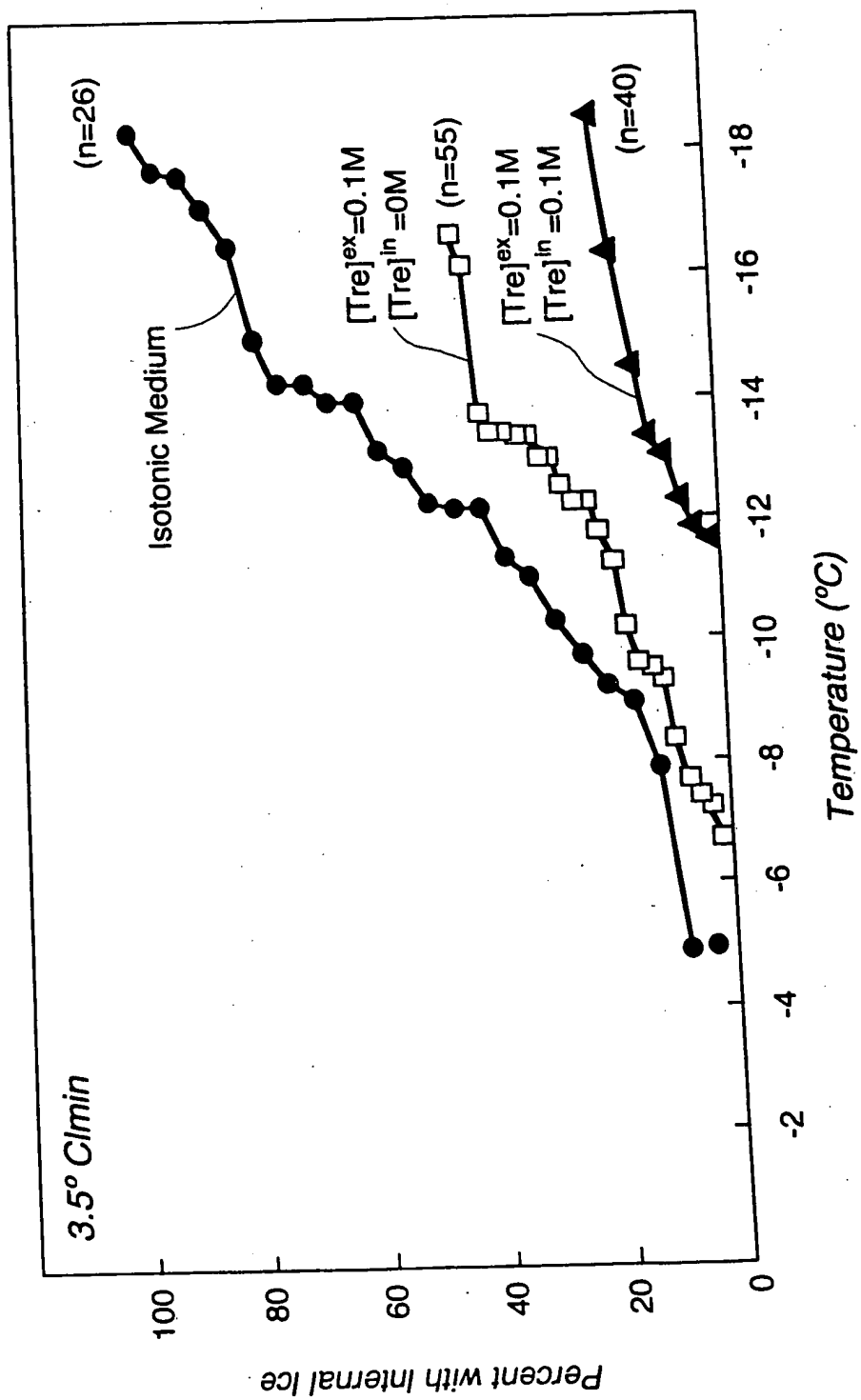

3.5° C/min
Isotonic Medium
(n=26)
[Tre]ex=0.1M
[Tre]in =0M
(n=55)
[Tre]ex=0.1M
[Tre]in =0.1M
(n=40)
Percent with Internal Ice
0
20
40
60
80
100
Temperature (°C)
-2
-4
-6
-8
-10
-12
-14
-16
-18

FIG. 13

FIG. 14

Solute	M_w	T_g, °C
erythrose	120.1	-50
threose	120.1	-45.5
erythritol	122.1	-53.5
thyminose(deoxyribose)	134.1	-52
ribulose	150.1	-50
xylose	150.1	-48
arabinose	150.1	-47.5
lyxose	150.1	-47.5
ribose	150.1	-47
arabitol	152.1	-47
ribitol	152.1	-47
xylitol	152.1	-46.5
methyl riboside	164.2	-53
methyl xyloside	164.2	-49
quinovose (deoxyglucose)	164.2	-43.5
fucose (deoxygalactase)	164.2	-43
rhamnose (deoxymannose)	164.2	-43
talose	180.2	-44
idose	180.2	-44
psicose	180.2	-44
altrose	180.2	-43.5
glucose	180.2	-43
gulose	180.2	-42.5
fructose	180.2	-42
galactose	180.2	-41.5

FIG. 14 CONT.

Solute	M_w	T_g ·C
allose	180.2	-41.5
sorbose	180.2	-41
mannose	180.2	-41
tagatose	180.2	-40.5
inositol	180.2	-35.5
mannitol	182.2	-40
galactitol	182.2	-39
sorbitol	182.2	-43.5
2-*O*-methyl fructoside	194.2	-51.5
β-1-*O*-methyl glucoside	194.2	-47
3-*O*-methyl glucoside	194.2	-45.5
6-*O*-methyl galactoside	194.2	-45.5
α-1-*O*-methyl glucoside	194.2	-44.5
1-*O*-methyl galactoside	194.2	-44.5
1-*O*-methyl mannoside	194.2	-43.5
1-*O*-ethyl glucoside	208.2	-46.5
2-*O*-ethyl fructoside	208.2	-46.5
1-*O*-ethyl galactoside	208.2	-45
1-*O*-ethyl mannoside	208.2	-43.5
glucoheptose	210.2	-37.5
mannoheptulose	210.2	-36.5
glucoheptulose	210.2	-36.5
perseitol (mannoheptitol)	212.2	-32.5
1-*O*-propyl glucoside	222.2	-43
1-*O*-propyl galactoside	222.2	-42

FIG. 14 CONT.

Solute	M_w	T_g°C
1-*O*-propyl mannoside	222.2	-40.5
2,3,4,6-*O*-methyl glucoside	236.2	-45.5
isomaltulose (palatinose)	342.3	-35.5
nigerose	342.3	-35.5
cellobiulose	342.3	-32.5
isomaltose	342.3	-32.5
sucrose	342.3	-32
gentiobiose	342.3	-31.5
laminaribiose	342.3	-31.5
turanose	342.3	-31
mannobiose	342.3	-30.5
melibiose	342.3	-30.5
lactulose	342.3	-30
maltose	342.3	-29.5
maltulose	342.3	-29.5
trehalose	342.3	-29.5
cellobiose	342.3	-29
lactose	342.3	-28
maltitol	344.3	-34.5
isomaltotriose	504.5	-30.5
panose	504.5	-28
raffinose	504.5	-26.5
maltotriose	504.5	-23.5
nystose	666.6	-26.5
stachyose	666.6	-23.5

FIG. 14 CONT.

Solute	M_w	T_g°C
maltotetraose	666.6	-19.5
maltopentaose	828.9	-16.5
α-cyclodextrin	972.9	-9
maltohexaose	990.9	-14.5
maltoheptaose	1153.0	-13.5

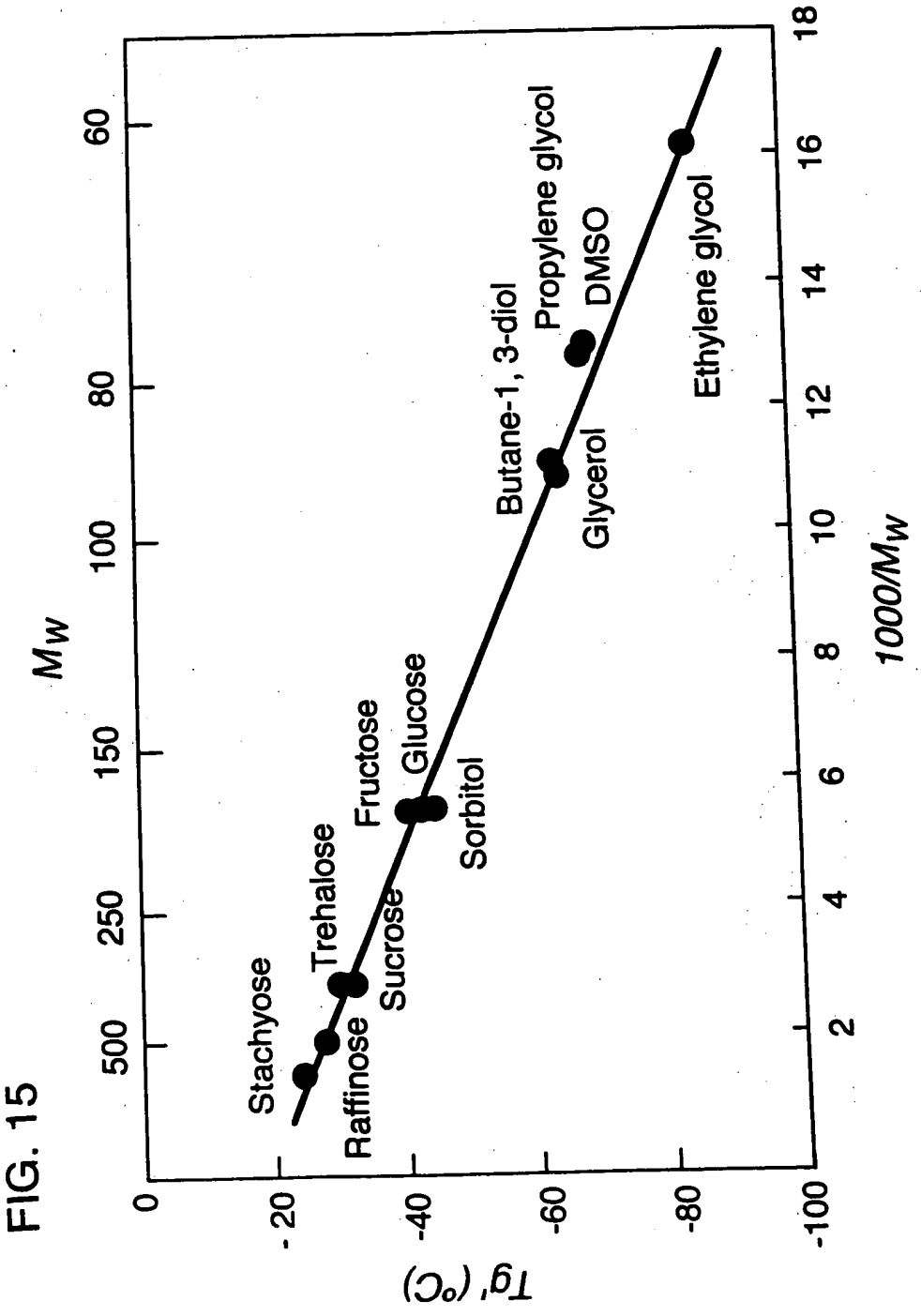

M_W
60
80
100
150
250
500
0
- 20
-40
-60
-80
-100
T_g' (°C)
2
4
6
8
10
12
14
16
18
1000/M_W
Stachyose
Raffinose
Trehalose
Sucrose
Fructose
Glucose
Sorbitol
Butane-1, 3-diol
Propylene glycol
DMSO
Glycerol
Ethylene glycol

FIG. 15

FIG. 16

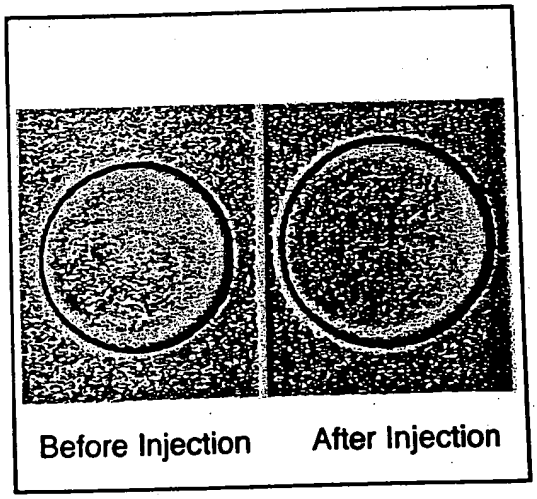


FIG. 17

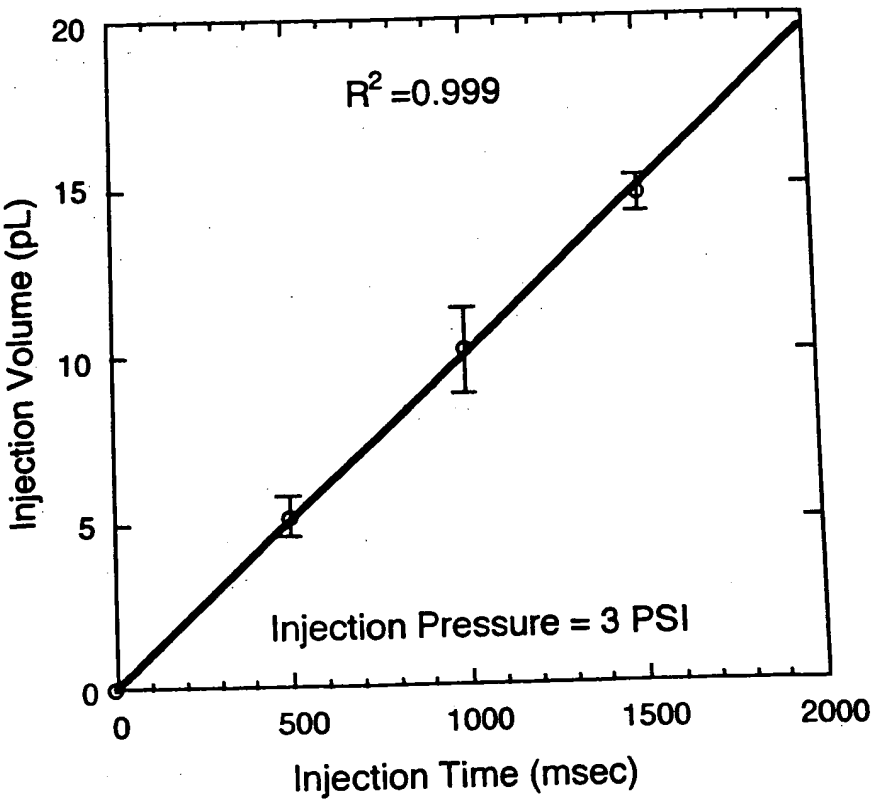

20
15
10
5
0
R^2 =0.999
Injection Pressure = 3 PSI
Injection Volume (pL)
0
500
1000
1500
2000
Injection Time (msec)

FIG. 18

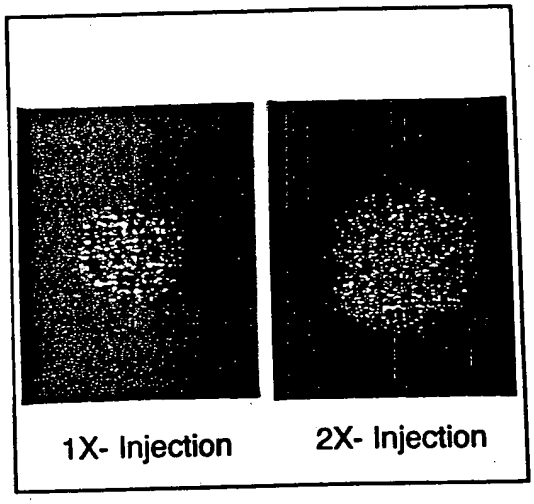


FIG. 19

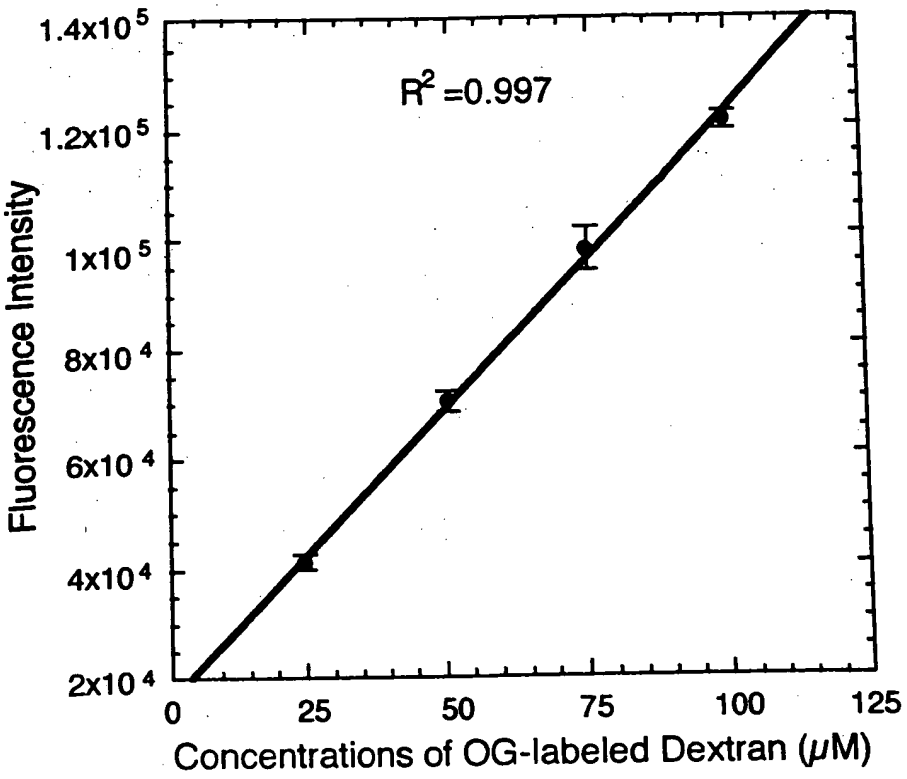

1.4x10 5
1.2x10 5
1x10 5
8x10 4
6x10 4
4x10 4
2x10 4
Fluorescence Intensity
R2 =0.997
0
25
50
75
100
125
Concentrations of OG-labeled Dextran (μM)

FIG. 20

Component	Hyper 320	Hyper 340	Hyper 360
NaCl *(mM)*	107.02	115.90	123.50
KCl *(mM)*	5.38	5.83	6.21
KH 2 PO_4 *(mM)*	0.43	0.46	0.49
$MgSO_4 \cdot 7H_2O$ *(mM)*	0.23	0.24	0.26
$NaHCO_3$ *(mM)*	28.16	30.50	32.50
$CaCl_2 \cdot 2H_2O$ *(mM)*	2.25	2.44	2.60
Lactate *(mM)*	22.53	24.40	26.00
Pyruvate *(mM)*	0.37	0.40	0.43
D- Glucose *(mM)*	3.13	3.39	3.61
L-Glutamine *(mM)*	1.13	1.22	1.30
EDTA *(mM)*	0.01	0.01	0.01
Phenol red *(mM)*	0.03	0.03	0.03
BSA *(mg/mL)*	4.00	4.00	4.00
Gentamicin *(mg/ml)*	50.00	50.00	50.00
Essential amino acids[i] *(mL/L)*	10.00	10.00	10.00
Non-essential amino acids[ii] *(mL/L)*	5.00	5.00	5.00
Osmolality	320	340	360

[i] *[50X Solution, Gibco]*
[ii] *[100X Solution, Gibco]*

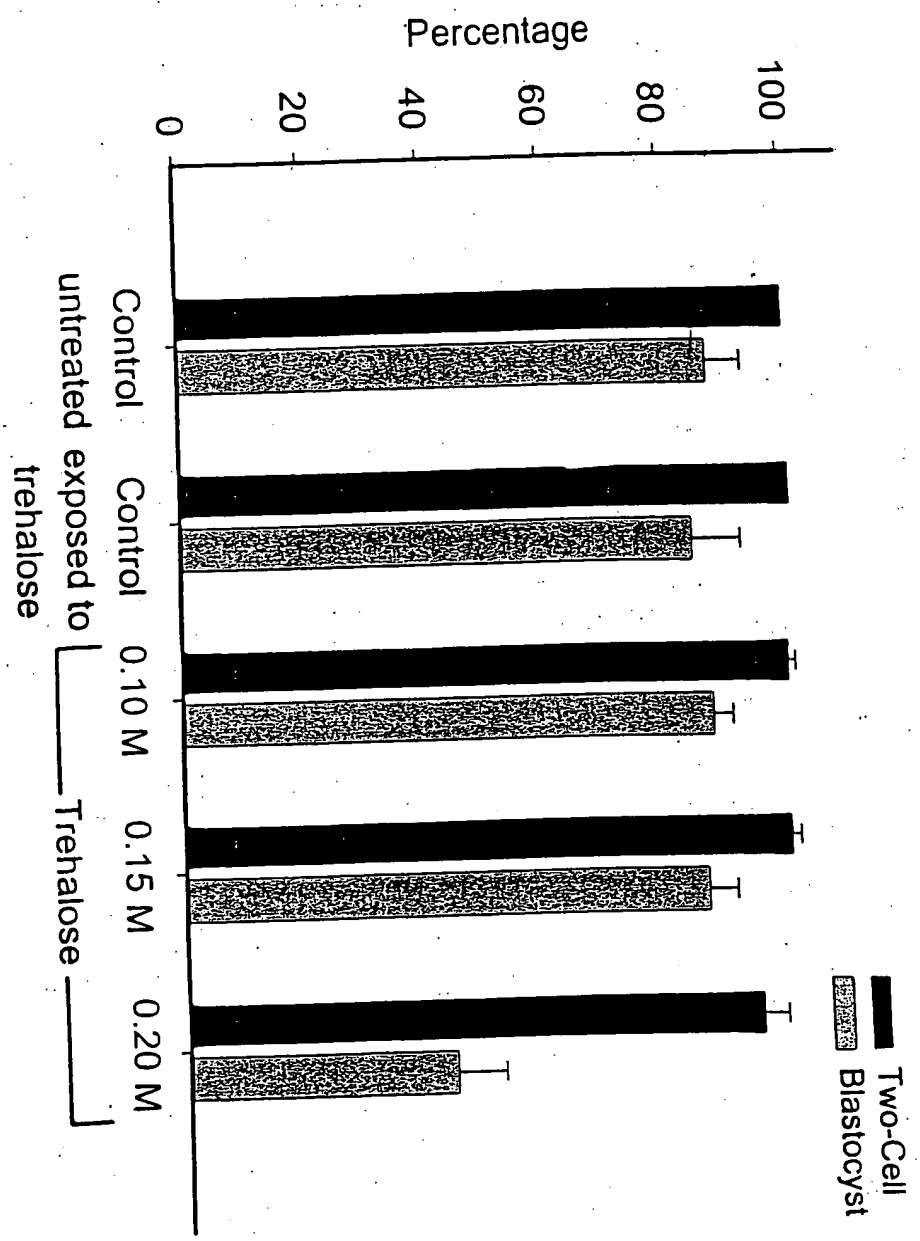

Percentage
0
20
40
60
80
100
Control
Control
0.10 M
0.15 M
0.20 M
untreated
exposed to
Trehalose
trehalose
Two-Cell
Blastocyst

Figure 21